Graphical Abstract

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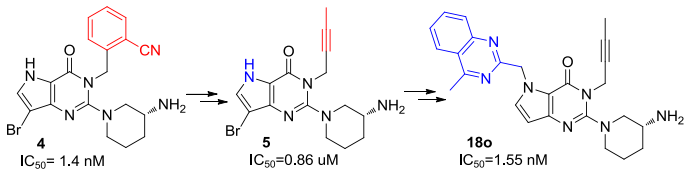

Discovery of potent dipeptidyl peptidase IV inhibitors through pharmacophore hybridization and hit-to-lead optimization

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ABSTRACT

A novel dipeptidyl peptidase IV inhibitor hit (**5**, IC_{50}= 0.86 μM) was structurally derived from our recently disclosed preclinical candidate **4** by replacing the cyanobenzyl with a butynyl based on pharmacophore hybridization. A hit-to-lead optimization effort was then initiated to improve its potency. Most N-substituted analogs exhibited good *in vitro* activity, and compound **18o** (IC_{50}= 1.55 nM) was identified to be a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor.

1. Introduction

Type 2 diabetes (T2D) formerly referred to as non-insulin-dependent or adult-onset diabetes, results from the body's ineffective use of insulin and comprises over 90% of diabetes patients. With more than 220 million people affected worldwide, diabetes has emerged as an epidemic, reflecting insufficient glycemic control and the urgency of more treatments[1]. Glucose like peptidase-1 (GLP-1) is an important incretin which contributes to the increase of insulin secretion and sensitivity, beta cell mass, and satiety, as well as the reduction of glucagon secretion and gastric emptying, which is helpful for glucose control for type 2 diabetics. However in the normal physical condition, GLP-1 is rapid truncated by dipeptidyl peptidase IV (DPP-IV) and lose function[2]. Thus inhibition of DPP-IV could effectively maintain the GLP-1 function and control glucose level. Compared to conventional anti-diabetic drugs, dipeptidyl peptidase IV (DPP-IV) inhibitors have good patient compliance, reduced risks of hypoglycemia, and less side effect[3]. As a result, first DPP-IV inhibitor occurred in the market in 2006 and gradually became the major intervention for type 2 diabetics.

Traditional medicinal strategies for protease inhibitors mostly rely on direct tight binding to the target, which leads to covalent compounds[4]. However, the emergence of non-covalent compounds to achieve satisfactory binding affinity with lower risk of selectivity issues has been witnessed in recent years[5]. Among the DPP-IV inhibitor, the first non-covalent DPP-IV inhibitor with good selectivity against DPP-8 and DPP-9[6], Sitagliptin (**1** Figure 1),[7] was marketed in 2006. Alogliptin (**2** Figure 1) was the second non-covalent DPP-IV inhibitor approved by the EMA, and it exhibited better efficacy than sitagliptin[8]. The most potent and long lasting drug is Linagliptin (**3** Figure 1) [9]. Other DPP-IV inhibitors include Vildagliptin[10], and Saxagliptin[11].

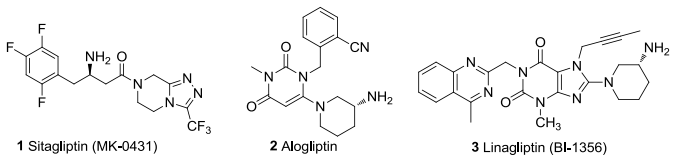

Figure 1. Marketed non-covalent DPP-IV inhibitors.

Our recently disclosed compound **4** was derived from Alogliptin by keeping its pharmacophore (3-aminopypiperidinyl region, red box, Figure 2) while modifying the scaffold[12]. **4** displayed a better in vivo efficacy than Alogliptin in lean mice and dose-dependent glucose reduction in T2D model ob/ob mice. Although **4** had a similar pharmacokinetic profile with Alogliptin in rat, yet 40% bioavailability and 2 hours half life are still not

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close to the requirements for an oral therapeutic candidate of a chronic disease. In our followed medicinal chemistry effort on **4**, we conducted several optimizations on it. Herein, we present our results about adoption of pharmacophore hybridization strategy in this process.

Close comparison of the pharmacophore of Linagliptin and compound **4** revealed that the cyanobenzyl should be freely changeable with butynyl if the 3-(R)-aminopiperidinyl group was still present. Thus, compound **5** was immediately generated based on pharmacophore hybridization. Though its activity was still in the micro molar range (IC_{50}= 0.86 μM), it could act as a starting point for further optimization.

The modification of compound **5** through N-substitution led to a series of novel DPP-IV inhibitors with significantly increased activity. The most active compound, **18o** (IC_{50}= 1.55 nM), was proved to be with much better pharmacokinetic profile than compound **4**, which implies potential better and competent in vivo efficacy for glucose control.

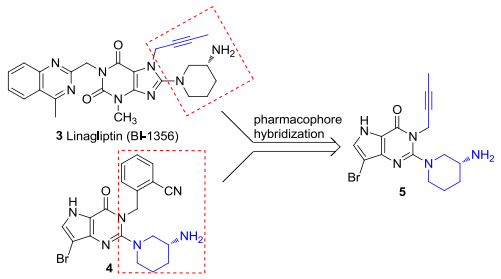
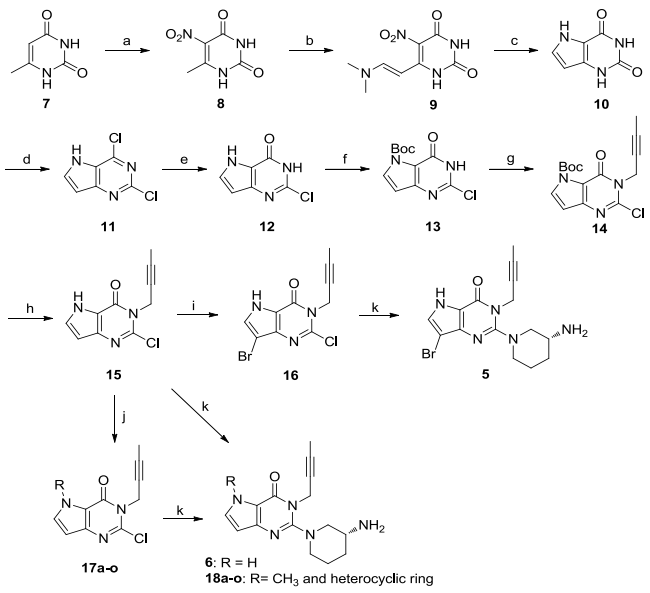


Figure 2. Generation of hit **5**.

2. chemistry

The synthesis of compounds **5**, **6** and **18a-o** is outlined in Scheme 1. Synthesis of compound **13** from **7** was described in our recent report[9]. Alkylation of **13** with 1-bromo-2-butyne provided precursor**14,** which was de-protected to give key intermediate **15**. The bromination of compound **15** with N-bromosuccinimide (NBS) provided compound**16**, which was converted to 5 by amination. The direct amination of **15** with3-(R)-aminopiperidine afforded compound 6. Compounds **18a-o** were obtained by the N-alkylation of 15 followed by the replacement of the chloro group with a 3-(R)-aminopiperidinyl group.



Scheme 1. Synthesis of compounds **5**, **6** and **18a-o**. Reagents: (a) sulfuric acid, fuming nitric acid, rt; (b) dimethylfomamide-dimethyl acetal, DMF, 80 ℃ then 140 ℃; (c) AcOH, Zn, 80 ℃; (d) $POCl_3$, DIEA, toluene, 70-80 ℃; (e) 1 N NaOH/H2O, 100 ℃; (f) $(Boc)_2O$, DMAP, Et_3N, THF, rt; (g) 1-bromo-2-butyne, DIEA, DMF, rt; (h) HCl/H_2O, MeOH, rt; (i) NBS, DCM, rt; (j)RX, NaH, DMF, rt; (k) 3-(R)-aminopiperidine, $NaHCO_3$, 120 ℃, ethanol.

3. Results and discussion

3.1. Hit identification through pharmacophore hybridization.

The similarity of the pharmacophore between Alogliptin, compound **4** and Linagliptin reminded us that the cyanobenzyl group of **4** could be replaced with a butynyl group without sacrificing *in vitro* activity (Figure 2). Because Linagliptin is the most potent and longest-lasting DPP-IV inhibitor on the market, we decided to test our hypothesis with a pharmacophore hybridization approach[10]. However, to our disappointment, compound 5 lost significant activity (IC_{50} = 0.86μM). Though this compound did not achieve the desired inhibition against DPP-IV, it might be a hit worthy of further optimization.

3.2. Hit-to-lead optimization on compound 5.

With compound 5 in hand, we immediately initiated a hit-to-lead optimization effort. First, compound **6**, without any substituent on the pyrolyl ring, was synthesized and found to have better activity with an IC_{50} of 0.46 μM. By simply adding a methyl group on the nitrogen (compound **18a**), the activity was further increased. This trend suggested that N-substitution might a powerful way to increase inhibition. Thus, we decided to make a series of N-substituted analogs (**18b-18o**).

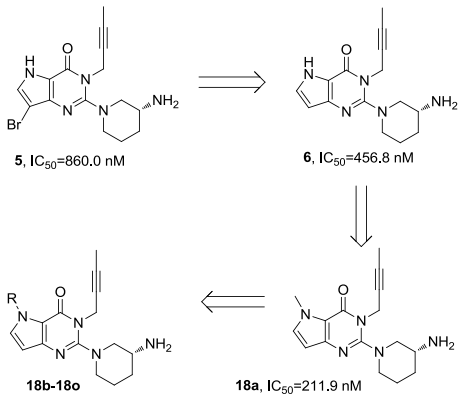
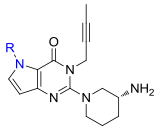


Figure 3. Hit-to-lead optimization strategy.

Though the first N-heterocyclic compound (**18b**) afforded further increased activity, it was still relatively inactive compared to parent compound **4** (IC_{50}= 44.0 nM). Inspired by the discovery of Linagliptin, where it was found that the N-5 position could hold larger steric substituents, a series of compounds with bicyclic rings at the N-position were made (**18c-o**). A few of these compounds displayed activity in the nM range, such as **18f**, **18l**, and **18m** (<10 nM). Surprisingly, the best compound (**18o**, IC_{50}= 1.55 nM) contained the same N-substituent as Linagliptin..

Table 1. Modifications at the N-5 position.



No.	R	DPP-IV[a]	No	R	DPP-IV[a]
18b		44.0	**18i**		14.5
18c		44.1	**18j**		52.6
18d		27.6	**18k**		11.4
18e		48.2	**18l**		4.67
18f		7.05	**18m**		5.93
18g		139.2	**18n**		15.5
18h		60.1	**18o**		1.55

[a]nM. Data represent the mean of at least three independent measurements.

*3.3. Biological evaluation of compound **18o**.*

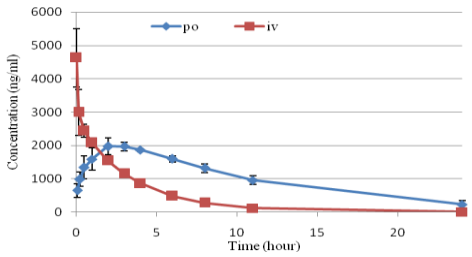


Figure 4. Concentration-time curve of **18o** in rat.

Compound **18o** was picked for preliminary evaluation for its high DPP-IV inhibitory activity. It had no inhibition against DPP-8 or 9 up to 10 μM. Compound **18o** had no CYP 3A inhibition up to 30 μM, which is likely to decrease the risk of drug-drug interactions. The pharmacokinetic study of **18o** also displayed a good drug-like profile and successfully overcame the insufficiency in the starting compound **4** (Table 2 and Figure 4). Compared to its parent compound **4**, it had a longer half life in rats (approximately 5 hours) and a better oral bioavailability (82.9%)that was comparable with marketed DPP-IV inhibitors (Alogliptin, 45%; Linagliptin, 50.7%) [9, 12-13]. Combined with the published data and current research progress of compound **4**, we believe that **18o** is highly possible to have a better in vivo efficacy than compound 4 and Alogliptin with doubled half life and bioavailability.

Table 2. pharmacokinetic parameters of **18o** in rat.

Dose iv/po	mg/kg	10/30
$AUC_{0-\infty}$ po	μg•h•mL^{-1}	25.3 ±2.6
$T_{1/2}$ po	hour	4.9±2.5v
Cl_z iv	L•h^{-1}•kg^{-1}	1.0±0.1
V_z iv	L•kg^{-1}	4.0±1.3
$MRT_{0-\infty}$ po	hour	8.3±1.6
F	%	82.9

po: oral administration. iv: intravenous injection.

4. Conclusion

In our followed medicinal chemistry effort of potent DPP-IV inhibitors based on our previously published compound **4**, we attempted several ways to explore the optimize space. Although compound 4 had a better in vivo efficacy than Alogliptin, its 40% bioavailability and 2 hours half life still unmet the requirements for oral chronic disease intervention. Among this process, inspired by the similarity in pharmacophores between Alogliptin, compound **4** and Linagliptin, we identified a novel DPP-IV inhibitor (**5**) by utilizing a pharmacophore hybridization strategy. Though the *in vitro* activity was still low, compound **5** (IC_{50}= 0.86 μM) was a logical hit for optimization and generated a series of DPP-IV inhibitors with significantly improved activity. Compound **18o** was finally obtained with the desired *in vitro* activity (IC_{50} = 1.55 nM) and much improved pharmacokinetic profile (bioavailability: 82.9%; half life: 4.9 hours). Thus **18o** had a high possibility of competent in vivo efficacy. Reviewed this part of work, we found the similarity between peer's work and ours[14]. Yet **18o** demonstrated a successful optimization course by adopting classical pharmacophore hybridization strategy. As one part of our followed optimization on compound **4**, more evaluations will be carried out on **18o** and other part of works will be prepared in due course.

5. Experimental section

5.1. Chemistry

All commercially available compounds and solvents were of reagent grade and used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang GF254 silica gel plates (5 x 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) and by spraying with an ethanol solution of 2,4-DNP or ninhydrin, or by fuming with iodine steam. Silica gel column chromatography was performed on silica gel (200-300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker NMR AVANCE 500 (500 MHz). Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). MS data were measured on an Agilent MSD-1200 ESI-MS system.

5.1.1. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (5)

A mixture of **16** (301 mg, 1.0 mmol), 3-(*R*)-aminopiperidine dihydrochloride (207mg, 1.2 mmol) and $NaHCO_3$ (336mg, 4.0 mmol) in a sealed tube containing 15 mL of ethanol was heated at 120 ℃ and stirred overnight. The reaction mixture was subsequently cooled to room temperature and filtered. The resulting filtrate was concentrated *in vacuo* and then purified by flash chromatography to yield compound**5**.Yield: 81.8%.^{1}HNMR (400MHz, MeOD) δ ppm: 7.37 (1H, s), 4.84-4.82 (2H, m), 3.53-3.50 (1H, m), 3.47-3.43 (1H, m), 3.06 (1H, s), 2.92-2.98 (1H, m), 2.03-2.00 (1H, m), 1.98-1.96 (1H, m), 1.89-1.87 (1H, m), 1.81 (3H, s),1.79-1.73 (1H, m), 1.33-1.27 (1H, m);^{13}CNMR (500 MHz, MeOD) δ ppm: 24.92 (1C), 28.68 (1C), 34.07 (1C),35.63 (1C), 52.68 (1C), 58.99 (1C), 75.29 (1C), 80.54 (1C), 81.04 (1C), 91.44 (1C), 116.26 (1C), 129.02 (1C), 142.13 (1C), 155.80 (1C), 156.49 (1C);ESI-MS calculated for ($C_{15}H_{19}N_5O$) $[M+H]^+$,363.07, 365.07, found 364.0, 366.0.

Compounds **6** and **18a-o** were prepared in a manner identical to that described for **5**.

5.1.2. 5.1.2 (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (6)

Yield: 67.8%. ^{1}HNMR (400MHz, CDCl3) δ ppm: 10.87 (1H, s), 7.23 (1H, d, J= 2.8 Hz), 6.36 (1H, d, J= 2.8 Hz), 4.85 (2H, s), 3.49-3.46 (1H, m), 3.36-3.33 (1H, m),3.11 (1H, m), 2.92 (1H, m), 2.80-2.78 (1H, m), 2.27 (2H, s), 1.99-1.97 (1H, m),1.88-1.86 (1H, m),1.79 (3H, s),1.73-1.70 (1H, m), 1.37-1.32 (1H, m);ESI-MS calculated for ($C_{15}H_{19}N_5O$) $[M+H]^+$, 286.16, found 286.1.

*5.1.3. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-methyl-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18a**)*

Yield: 52.6%. ^{1}HNMR (400MHz, CDCl3) δ ppm: 6.87 (1H, d, J= 2.8 Hz), 6.17 (1H, d, J= 2.8 Hz), 5.19 (2H, s), 4.77-4.63 (2H, AB q, J= 34.8 Hz, 16.4 Hz), 3.96 (1H, s), 3.50-3.47 (1H, m), 3.32 (2H, m),3.03-2.98 (1H, m), 2.90 (1H, m), 2.05 (1H, m), 1.84 (1H, m), 1.75 (3H, s),1.65-1.63 (2H, m);^{13}CNMR (500 MHz, CDCl3) δ ppm: 22.64 (1C), 28.34 (1C), 30.38 (1C),34.41 (1C), 35.57 (1C),47.69 (1C), 51.55 (1C), 55.40 (1C), 74.46 (1C), 79.35 (1C),101.61 (1C), 115.10 (1C), 131.69 (1C), 142.73 (1C), 153.49 (1C), 155.42 (1C); ESI-MS calculated for (C16H21N5O) $[M+H]^+$, 300.17, found 300.1.

*5.1.4. (R)-2-(2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-4-oxo-3H-pyrrolo[3,2-d]pyrimidin-5(4H)-yl)acetonitrile(**18b**)*

Yield: 54.2%. ^{1}HNMR (400MHz, CDCl3) δ ppm: 7.14 (1H, d, J= 2.8 Hz), 6.39 (1H, d, J= 2.8 Hz), 5.46 (2H, s), 4.76 (2H, s), 3.49-3.47 (1H, m), 3.38-3.35 (1H, m), 3.15 (1H, m), 2.96-2.91 (1H, m), 2.80 (2H, s), 2.00-2.96 (1H, m), 1.88-1.84 (1H, m), 1.80 (3H, s),1.75-1.65 (2H, m), 1.42 (1H, m);13CNMR (500 MHz, CDCl3) δ ppm: 22.95 (1C), 28.35 (1C), 32.54 (1C),34.80 (1C), 35.47 (1C),47.58 (1C), 50.39 (1C), 51.26 (1C),58.04 (1C), 73.97 (1C), 79.86 (1C),104.73 (1C), 114.60 (1C), 130.28 (1C), 143.98 (1C), 154.66 (1C), 155.57 (1C); ESI-MS calculated for (C17H20N6O) $[M+H]^+$, 325.17, found 325.1.

*5.1.5. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-(pyrimidin-2-ylmethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18b**)*

Yield: 54.5%. 1HNMR (400MHz, MeOD) δ ppm: 8.65-8.63 (2H, d, J= 4.8 Hz), 7.32 (1H, d, J= 2.8 Hz), 7.31-7.28 (1H, t, J= 4.8 Hz), 6.31 (1H, d, J= 2.8 Hz), 5.81 (2H, s), 4.76-4.67 (2H, q, J= 18.8 Hz), 3.46-3.43 (1H, m), 3.36-3.29 (1H, m), 3.12-3.08 (1H, m), 2.90 (1H, m), 2.81 (1H, m), 2.78-2.76 (1H, m), 2.01-1.98 (1H, m), 1.88-1.86 (1H, m), 1.85-1.75 (1H, m), 1.72 (3H, s), 1.43-1.39 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 22.34 (1C), 28.87 (1C), 32.60 (1C),35.16 (1C), 49.51 (1C),50.51 (1C), 52.77 (1C),54.39 (1C), 58.06 (1C), 75.36 (1C), 80.41 (1C),103.23 (1C), 116.32 (1C), 121.15 (1C), 134.41 (1C), 145.10 (1C), 155.54 (1C), 156.79 (1C), 158.70 (1C), 167.68 (1C); ESI-MS calculated for (C20H23N7O) $[M+H]^+$, 378.20, found 378.1.

*5.1.6. (R)-5-((1H-indol-3-yl)methyl)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18c**)*

Yield: 51.7%. 1HNMR (400MHz, MeOD) δ ppm: 7.46-7.44 (1H, d, J= 8.0 Hz), 7.30 (1H, s), 7.28 (1H,s), 7.28 (1H, d, J= 2.8 Hz), 7.04-7.00 (1H, m), 6.92-6.88 (1H, m), 6.13 (1H, d, J= 2.8 Hz), 5.73 (2H, s), 4.81 (2H, s), 3.57 (1H, m), 3.47-3.44 (1H, m), 3.26-3.25 (1H, m),3.07-3.01 (1H, m), 2.05 (1H, m), 1.89-1.87 (1H, m), 1.78 (1H, m), 1.77 (3H, s), 1.63-1.61 (1H, m), 1.22 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 22.47 (1C), 28.92 (1C), 35.44 (1C), 44.25 (1C), 48.49 (1C), 49.51 (1C), 52.92 (1C),53.85 (1C), 58.06 (1C), 61.44 (1C), 75.36 (1C), 80.41 (1C),102.73 (1C), 112.44 (1C),115.73 (1C), 119.23 (1C), 120.29 (1C), 122.77 (1C), 125.81 (1C), 127.80 (1C), 133.07 (1C), 138.14 (1C),144.66 (1C),154.56 (1C), 156.82 (1C); ESI-MS calculated for (C24H26N6O) $[M+H]^+$, 415.22, found 415.1.

*5.1.7. (R)-5-((1H-benzo[d]imidazol-2-yl)methyl)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18d**)*

Yield: 77.1%. 1HNMR (400MHz, MeOD) δ ppm: 7.47 (2H, m), 7.39 (1H, d, J= 2.8 Hz), 7.18-7.15 (2H, m), 6.32 (1H, d, J= 2.8 Hz), 5.84 (1H, s), 4.78 (2H, s), 3.46-3.44 (1H, m), 3.43-3.42 (1H, m), 3.41-3.40 (1H, m),3.03-2.98 (1H, m), 2.08-2.05 (1H, m), 1.91-1.88 (1H, m), 1.82-1.79 (1H, m), 1.77-1.75 (1H, m), 1.73 (3H, s), 1.60-1.57 (1H, m);ESI-MS calculated for (C23H25N7O) $[M+H]^+$, 416.21, found 416.2.

*5.1.8. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-(quinolin-4-ylmethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18e**)*

Yield: 51.6%. 1HNMR (400MHz, MeOD) δ ppm: 8.67 (1H, d, J= 8.0 Hz), 8.25-8.23 (1H, d, J= 8.0 Hz), 8.08-8.06 (1H, d, J= 8.0 Hz), 7.85-7.81 (1H, m), 7.73-7.69 (1H, m), 7.40 (1H, d, J= 2.8 Hz), 6.62 (1H, d, J= 2.8 Hz), 6.43-6.42 (1H, m), 6.24 (2H, s), 4.79-4.70 (2H, t, J= 19.2 Hz), 3.48-3.45 (1H, m), 3.41-3.38 (1H, m), 3.03-2.98 (1H, m), 2.93-2.87 (1H, m), 2.76-2.70 (1H, m), 2.01-1.98 (1H, m), 1.90-1.87 (1H, m), 1.79-1.76 (1H, m), 1.74 (3H, s), 1.40-1.30 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 24.52 (1C), 33.74 (1C), 35.42 (1C), 48.53 (1C), 49.55 (1C), 49.58 (1C), 52.76 (1C),59.35 (1C), 75.28 (1C), 80.52 (1C), 103.96 (1C), 116.09 (1C),119.10 (1C), 124.29 (1C),127.06 (1C), 128.60 (1C), 129.89 (1C), 131.22 (1C), 134.10 (1C), 145.54 (1C), 147.43 (1C), 148.48 (1C),151.30 (1C), 156.14 (1C), 156.94 (1C); ESI-MS calculated for (C25H26N6O) $[M+H]^+$, 427.22, found 427.1.

*5.1.9. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-(quinolin-2-ylmethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18f**)*

Yield: 54.8%. 1HNMR (400MHz, CDCl3) δ ppm: 8.20-8.18 (1H, d, J= 8.0 Hz), 7.98-7.96 (1H, d, J= 8.8 Hz), 7.83-7.81 (1H, d, J= 8.0 Hz), 7.85-7.81 (1H, m), 7.73-7.70 (1H, m), 7.54-7.51 (1H, m), 7.40 (1H, d, J= 2.8 Hz), 6.35 (1H, d, J= 2.8 Hz), 5.87 (2H, s), 4.79-4.69 (2H, t, J= 21.6 Hz), 3.44-3.41 (1H, m), 3.36-3.29 (1H, m), 2.99-2.94 (1H, m), 2.88-2.85 (1H, m), 2.71-2.63 (1H, m), 1.99-1.95 (1H, m), 1.86-1.83 (1H, m), 1.73 (1H, m), 1.73 (3H, s), 1.38-1.26 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 24.48 (1C), 33.69 (1C), 35.32 (1C), 48.48 (1C), 49.51 (1C), 52.69 (1C), 54.44 (1C),59.33 (1C), 75.36 (1C), 80.43 (1C), 103.79 (1C), 115.92 (1C),120.19 (1C), 127.82 (1C),128.85 (1C), 128.94 (1C), 129.19 (1C), 131.24 (1C), 133.92 (1C), 139.13 (1C), 145.41 (1C), 148.50 (1C),155.96 (1C), 156.92 (1C), 159.53 (1C); ESI-MS calculated for (C25H26N6O) $[M+H]^+$, 427.22, found 427.1.

*5.1.10. (R)-2-(3-aminopiperidin-1-yl)-5-((6-bromoquinolin-2-yl)methyl)-3-(but-2-yn-1-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18g**)*

Yield: 43.8%. 1HNMR (400MHz, MeOD) δ ppm: 8.14-8.11 (1H, d, J= 8.8 Hz), 8.00-7.99 (1H, d, J= 2.0 Hz), 7.90-7.87 (1H, m), 7.80-7.77 (1H, m), 7.44 (1H, d, J= 2.8 Hz), 7.23-7.21 (1H, d, J= 8.8 Hz), 6.42 (1H, d, J= 2.8 Hz), 5.91 (2H, s), 4.83-4.81 (2H, m), 3.53-3.50 (1H, m), 3.45-3.42 (1H, m), 3.10-3.04 (1H, m),

2.96-2.91 (1H, m), 2.80-2.75 (1H, m), 2.07-2.03 (1H, m), 1.95-1.90 (1H, m), 1.84 (3H, s), 1.83-1.75 (1H, m), 1.45-1.33 (1H, m);13CNMR (500 MHz, CDCl3+MeOD) δ ppm: 24.27 (1C), 33.45 (1C), 35.29 (1C), 48.48 (1C), 49.50 (1C), 49.98 (1C), 52.50 (1C), 54.29 (1C),59.07 (1C), 75.14 (1C), 80.40 (1C), 103.70 (1C), 115.68 (1C),121.17 (1C), 129.63 (1C),130.72 (1C), 131.04 (1C), 133.61 (1C), 134.19 (1C), 137.85 (1C), 145.06 (1C), 146.83 (1C), 155.63 (1C), 156.67 (1C), 159.63 (1C); ESI-MS calculated for (C25H25BrN6O) $[M+H]^+$, 505.13, found 505.1.

*5.1.11. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((6-chloroquinolin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18h**)*

Yield: 67.8%. 1HNMR (400MHz, CDCl3) δ ppm: 7.96-7.93 (1H, m), 7.71 (1H, d, J= 2.4 Hz), 7.60-7.57 (1H, dd, J= 2.4 Hz, J= 9.2 Hz), 7.30-7.26 (1H, m), 7.20 (1H, d, J= 2.8 Hz), 6.34 (1H, d, J= 2.8 Hz), 5.88 (2H, s), 4.79 (2H, d, J= 2.0 Hz), 3.48-3.43 (1H, m), 3.06-3.00 (1H, m), 2.90-2.85 (1H, m), 2.72-2.67 (1H, m), 1.98-1.93 (1H, m), 1.89 (2H, s), 1.85-1.82 (1H, m), 1.80 (3H, s), 1.74-1.65 (1H, m), 1.32-1.24 (1H, m);13CNMR (500 MHz, CDCl3) δ ppm: 23.41 (1C), 33.45 (1C), 34.49 (1C), 47.83 (1C), 51.36 (1C), 53.67 (1C), 59.26 (1C), 74.47 (1C), 77.00 (1C), 79.34 (1C), 103.12 (1C), 114.75 (1C),120.82 (1C), 126.14 (1C),127.93 (1C), 130.52 (1C), 130.74 (1C), 131.49 (1C), 132.14 (1C), 136.21 (1C), 143.72 (1C), 145.95 (1C), 154.24 (1C), 155.72 (1C), 158.00 (1C); ESI-MS calculated for (C25H25ClN6O) $[M+H]^+$, 461.18, found 461.1.

*5.1.12. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((6-fluoroquinolin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18i**)*

Yield: 62.5%. 1HNMR (400MHz, CDCl3) δ ppm: 8.02-7.97 (2H, m), 7.46-7.41 (1H, m), 7.36-7.33 (1H, m), 7.31-7.29 (1H, d, J= 8.4 Hz), 7.20 (1H, d, J= 2.8 Hz), 6.33 (1H, d, J= 2.8 Hz), 5.89 (2H, s), 4.79 (2H, d, J= 2.0 Hz), 3.48-3.43 (1H, m), 3.37-3.34 (1H, m), 3.06-3.00 (1H, m), 2.90-2.85 (1H, m), 1.98-1.93 (1H, m), 1.87-1.84 (1H, m), 1.83 (2H, s), 1.79 (3H, s), 1.74-1.66 (1H, m), 1.32-1.24 (1H, m);13CNMR (500 MHz, CDCl3) δ ppm: 23.40 (1C), 33.45 (1C), 34.45 (1C), 47.81 (1C), 51.34 (1C), 53.63 (1C), 59.26 (1C), 74.48 (1C), 77.00 (1C), 79.31 (1C), 103.06 (1C), 110.37 (1C),114.73 (1C), 119.68 (1C),120.72 (1C), 127.90 (1C), 131.53 (1C), 136.45 (1C), 143.70 (1C), 144.63 (1C), 154.21 (1C), 155.70 (1C), 157.00 (1C), 159.34 (1C), 161.31 (1C); ESI-MS calculated for (C25H25FN6O) $[M+H]^+$, 445.21, found 445.1.

*5.1.13. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((6-methylquinolin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one(**18j**)*

Yield: 69.4%. 1HNMR (400MHz, MeOD) δ ppm: 8.06-8.04 (1H, d, J= 8.4 Hz), 7.86-7.84 (1H, d, J= 8.4 Hz), 7.62 (1H, s), 7.61-7.59 (1H, dd, J= 8.8 Hz, J= 1.6 Hz), 7.39 (1H, d, J= 2.8 Hz), 7.30-7.28 (1H, d, J= 8.4Hz), 6.34 (1H, d, J= 2.8 Hz), 5.73 (2H, s), 4.80-4.74 (2H, m), 3.45-3.43 (1H, m), 3.35-3.34 (1H, m), 3.01-2.95 (1H, m), 2.87-2.67 (1H, m), 2.65 (3H, s), 2.00-1.96 (1H, m), 1.88-1.84 (1H, m), 1.77 (3H, s), 1.74-1.66 (1H, m), 1.38-1.35 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 24.63 (1C), 33.73 (1C), 35.33 (1C), 48.49 (1C), 49.51 (1C), 52.19 (1C), 52.26 (1C), 55.50 (1C), 59.40 (1C), 75.49 (1C), 80.41 (1C), 103.56 (1C), 115.64 (1C),123.60 (1C), 127.07 (1C),127.79 (1C), 128.74 (1C), 130.34 (1C), 133.41 (1C), 137.70 (1C), 138.21 (1C), 145.28 (1C), 147.72 (1C), 155.81 (1C), 156.77 (1C), 160.52 (1C); ESI-MS calculated for (C26H28N6O) $[M+H]^+$, 441.23, found 441.1.

*5.1.14. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((7-chloroquinolin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18k**)*

Yield: 43.4%. 1HNMR (400MHz, CDCl3) δ ppm: 8.03 (1H, d, J= 1.6 Hz), 8.01 (1H, s), 7.69-7.67 (1H, d, J= 8.8 Hz), 7.45-7.43 (1H, dd, J= 8.8 Hz, J= 2.0 Hz), 7.30-7.28 (1H, d, J= 8.8 Hz), 7.22 (1H, d, J= 2.8 Hz), 6.35 (1H, d, J= 2.8 Hz), 5.90 (2H, s), 4.84-4.75 (2H, m), 3.50-3.47 (1H, m), 3.37-3.34 (1H, m), 3.16-3.12 (1H, m), 2.97-2.92 (1H, m), 2.84-2.80 (1H, m), 2.43(2H, s), 1.99-1.96 (1H, m), 1.89-1.85 (1H, m), 1.80 (3H, t, J= 2.4 Hz), 1.76-1.66 (1H, m), 1.42-1.40 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 23.07 (1C), 29.65 (1C), 32.65 (1C), 34.53 (1C), 47.70 (1C), 51.40 (1C), 53.67 (1C), 58.27 (1C), 74.45 (1C), 79.46 (1C), 103.11 (1C), 114.78 (1C), 120.14 (1C),125.74 (1C), 127.53 (1C),128.21 (1C), 128.71 (1C), 131.64 (1C), 135.49 (1C), 136.98 (1C), 143.59 (1C), 147.96 (1C), 154.12 (1C), 155.67 (1C), 158.74 (1C); ESI-MS calculated for (C25H25ClN6O) $[M+H]^+$, 461.18, found 461.1.

*5.1.15. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((7-fluoroquinolin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18l**)*

Yield: 45.9%. 1HNMR (400MHz, CDCl3) δ ppm: 8.04-8.02 (1H, d, J= 8.4 Hz), 7.75-7.64 (1H, m), 7.67-7.64 (1H, dd, J= 5.2 Hz, J= 2.0 Hz), 7.30-7.28 (1H, m), 7.24 (1H, s), 7.22 (1H, d, J= 2.8 Hz), 6.35 (1H, d, J= 2.8 Hz), 5.90 (2H, s), 4.80 (2H, d, J= 2.0 Hz), 3.49-3.47 (1H, m), 3.38-3.35 (1H, m), 3.07 (1H, m), 2.93-2.88 (1H, m), 2.76-2.71 (1H, m), 1.98-1.95 (1H, m), 1.88 (1H, m), 1.85 (2H, s), 1.80 (3H, s), 1.76-1.66 (1H, m), 1.33-1.31 (1H, m);13CNMR (500 MHz, CDCl3) δ ppm: 23.17 (1C), 33.51 (1C), 34.49 (1C), 47.03 (1C), 51.37 (1C), 53.71 (1C), 74.51 (1C), 79.34 (1C), 103.12 (1C), 112.76 (1C), 114.80 (1C), 116.87 (1C), 119.24 (1C),124.38 (1C), 129.45 (1C),131.53 (1C), 137.02 (1C), 143.70 (1C), 148.55 (1C), 154.22 (1C), 155.73 (1C), 158.81 (1C), 162.13 (1C), 164.12 (1C); ESI-MS calculated for (C25H25FN6O) $[M+H]^+$, 445.21, found 445.1.

*5.1.16. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-(quinoxalin-2-ylmethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one(**18m**)*

Yield: 61.5%. 1HNMR (400MHz, MeOD) δ ppm: 8.67 (1H, s), 7.97-7.90 (2H, m), 7.74-7.69 (2H, m), 7.45 (1H, d, J= 2.8 Hz), 6.35 (1H, d, J= 2.8 Hz), 5.90 (2H, s), 4.78-4.71 (2H, m), 3.44-3.41 (1H, m), 3.36-3.32 (1H, m), 2.99-2.95 (1H, m), 2.86-2.84 (1H, m), 2.71-2.66 (1H, m), 1.99-1.94 (1H, m), 1.85-1.82 (1H, m), 1.73 (3H, s), 1.69-1.68 (1H, m), 1.37-1.26 (1H, m);13CNMR (500 MHz, MeOD) δ ppm: 24.46 (1C), 33.68 (1C), 35.33 (1C), 48.48 (1C), 49.98 (1C), 52.64 (1C), 54.40 (1C), 59.33 (1C), 75.35 (1C), 80.43 (1C), 103.71 (1C), 115.75 (1C), 129.67 (1C), 129.98 (1C), 131.16 (1C),131.58 (1C), 134.10 (1C),142.64 (1C), 142.93 (1C), 145.16 (1C), 145.45 (1C), 154.23 (1C), 155.90 (1C), 156.81 (1C); ESI-MS calculated for (C24H25N7O) $[M+H]^+$, 428.21, found428.1.

*5.1.17. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((3-methylquinoxalin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (**18n**)*

Yield: 53.4%. 1HNMR (400MHz, CDCl3) δ ppm: 7.98-7.92 (2H, m), 7.69-7.61 (2H, m), 7.07 (1H, d, J= 2.8 Hz), 6.34 (1H, d, J= 2.8 Hz), 6.04 (2H, s), 4.78 (2H, d, J= 2.0 Hz), 3.49-3.46 (1H, m), 3.38-3.35 (1H, m), 3.06-3.01 (1H, m), 2.91-2.86 (1H, m), 2.72 (3H, s), 2.72 (1H, m), 1.98-1.94 (1H, m), 1.87-1.81 (1H, m), 1.77 (2H, s), 1.77 (3H, s), 1.72-1.68 (1H, m), 1.29-1.27 (1H, m);13CNMR (500 MHz, CDCl3) δ ppm: 22.13 (1C), 23.44 (1C), 29.63 (1C), 33.46 (1C), 34.48 (1C), 47.84 (1C), 50.93 (1C), 51.37 (1C), 59.32 (1C), 74.46 (1C), 76.78 (1C), 79.35 (1C), 103.08 (1C), 115.00 (1C), 128.29 (1C), 128.98 (1C), 129.81 (1C),131.41 (1C), 140.66 (1C),141.61 (1C), 143.54 (1C), 150.83 (1C), 152.80 (1C), 154.17 (1C), 155.84 (1C); ESI-MS calculated for (C25H27N7O) $[M+H]^+$, 442.23, found442.2.

*5.1.18. (R)-2-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-5-((4-methylquinazolin-2-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one(**18o**)*

Yield: 59.0%. 1HNMR (400MHz, CDCl3) δ ppm: 7.97-7.95 (2H, d, J= 8.4 Hz), 7.77-7.70 (2H, m), 7.51-7.47 (1H, m), 7.13 (1H, d, J= 2.8 Hz), 6.28 (1H, d, J= 2.8 Hz), 5.86 (2H, s), 4.68-4.58 (2H, dd, J= 33.6 Hz, J= 4.0 Hz), 3.85 (2H, s), 3.39-3.37 (1H, m), 3.27-3.24 (1H, m), 3.06 (1H, m), 2.85 (1H, m), 2.79 (3H, s), 2.75-2.73 (1H, m), 1.93-1.90 (1H, m), 1.80-1.76 (1H, m), 1.64 (3H, s), 1.64 (1H, m),1.36-1.34 (1H, m);13CNMR (500 MHz, CDCl3 + MeOD) δ ppm: 21.24 (1C), 22.30 (1C), 31.24 (1C), 34.09 (1C), 47.11 (1C), 49.02 (1C), 51.32 (1C), 53.40 (1C), 56.65 (1C), 74.01 (1C), 79.28 (1C), 102.17 (1C), 115.14 (1C), 122.87 (1C), 124.82 (1C), 127.31 (1C),128.35 (1C), 132.27 (1C),133.72 (1C),142.95 (1C),149.42 (1C),153.76 (1C),155.34 (1C),160.96 (1C),169.08 (1C);ESI-MS calculated for (C25H27N7O) $[M+H]^+$, 442.23, found442.2

5.2. In vitro inhibition of DPP-IV, DPP-8 and DPP-9

Solutions of test compounds at varying concentrations (≤10 mM final concentration) were prepared in dimethyl sulfoxide (DMSO) and diluted into assay buffer containing 20 mM Tris (pH 7.4), 20 mM KCl, and 0.1mg/mL BSA. Human DPP-IV (0.1 nM final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated by the addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin, Sigma-Aldrich, 10μM final concentration). The total volume of the reaction mixture was 100 μL. The kinetics of the reaction was monitored (excitation at 400 nm, emission at 505 nm) for 5-10 minutes, or an endpoint was measured after 10 minutes. Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models

5.3. In vivo pharmacokinetic study

Adult male SD rats (n= 4/group) were administered the test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg for oral administration and 5 mg/mL by injection. Blood samples of 100-200 μL were collected from the orbit at 11 time points within 24 hours. The blood concentration of test compounds was determined by LC-MS/MS. The PK parameters were obtained from the pharmacokinetic software DAS. 2.0.

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